PATENT
Docket No. 290.0001 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Regnier et al.	Group Art Unit:	1639
Serial No.:	09/849,924	Examiner:	Tran
Confirmation No.:	8955		
Filed:	4 May 2001		

For: AFFINITY SELECTED SIGNATURE PEPTIDES FOR PROTEIN IDENTIFICATION AND QUANTIFICATION

DECLARATION UNDER 37 C.F.R. §1.131

Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Fred E. Regnier, Ph.D., declare and say as follows:

1. I am the sole inventor of the subject matter of claims 1-32, presently pending in the above-identified U.S. Patent Application Serial No. 09/849,924, filed May 4, 2001.

2. I received a Ph.D. in Chemistry 1965 from Oklahoma State University in Stillwater, OK, and a B.S. in Chemistry from Nebraska State College, Peru, NE. From 1965-1968 I worked as a Post-Doctoral Associate at Oklahoma State University, the University of Chicago, Chicago, IL, and Harvard University, Cambridge, MA. I joined Purdue University in 1968 as Assistant Professor of Biochemistry, was appointed Professor of Biochemistry in 1976, and have been Professor of Chemistry at Purdue University since 1990. My research activities presently focus on chromatographic and mass spectrometric advances in proteomics, and also "chemistry-on-a-chip" technologies. I have been a member of the editorial boards of a number of scientific journals, and have published over 200 papers on these and related topics.

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3. The instant application claims the benefit of U.S. Provisional Patent Applications Serial No. 60/203,227, filed May 5, 2000; Serial No. 60/208,372, filed May 31, 2000; and Serial No. 60/208,184, filed May 31, 2000.

4. I have reviewed the above-entitled U.S. Patent Application, the U.S. Provisional Patent Application priority documents referenced herein, and the Office Action mailed June 17, 2003, as well as U.S. Patent No. 6,391,649 (Chait et al.) and Gygi et al. (*Nature Biotechnology*, *17*:994-999 (October 1, 1999)) cited therein. I make this declaration in support of the patentability of the claims of the above-identified application.

5. Prior to May 4, 1999, I conceived of and reduced to practice a method for analyzing genetic expression by identifying proteins that are up-regulated or down-regulated using mass spectrometry of isotopically labeled samples. This invention is evidenced by my manuscript entitled "A Strategy for the Labeling and Selective Analysis of Cysteine, Tryptophan, Methionine, Histidine and Tyrosine Containing Peptide Fragments As a Round to the Analysis of Complex Protein Mixtures" (Exhibit A), and by my grant application entitled "Chip Based Systems for the Analysis of Regulation: A New Dimension in Proteomics" (Exhibit B). The manuscript (Exhibit A) has been redacted to delete the signature and date of a witness (one of my students), which date is prior to May 4, 1999. The grant application (Exhibit B) has been redacted to remove an extraneous telephone number written on the fourth page of the exhibit, and to delete dates indicated at the first, seventh, and the thirty third page. The grant application, (Exhibit B) was written and submitted to the Public Health Service prior to May 4, 1999.

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6. Exhibit A describes, at pages A-4 and A-5 under the subheading "Analysis of Genetic Expression," methods for detecting a difference in the concentration of a protein present in a first sample and in a second sample that include labeling proteins in first and second samples after biosynthesis with first and second isotopic variants of a single chemical moiety (e.g., by using isotopic variants of an affinity tag used for cysteine), mixing of the labeled samples, optional cleavage of the sample proteins to yield peptides, and mass spectrometric analysis of the labeled proteins or peptides. Affinity selection, electrophoretic separation and chromatographic separation of the labeled species are also described.

7. Exhibit B describes, at page B-22 and B-23 under the heading "Isotopic Labeling," isotopically labeling the peptides in first and second samples (e.g., through labeling primary amines) after biosynthesis with first and second isotopic variants of a single chemical moiety (e.g., using normal or deuterated acetyl N-hydroxysuccinimide) after fragmentation but before mixing and mass spectrometric analysis. Further, at page B-21 (last paragraph) Exhibit B describes that normal and heavy isotopic forms of a protein cannot be resolved using a separation system, but can be differentiated, either as proteolytic fragments or in the whole protein, using a mass spectrometer.

8. Support for the present invention is found in the priority document U.S. Provisional Patent Application 60/203,227, filed May 5, 2000. For example, at pages 1-17 the isotopic labeling of proteins is disclosed. Labeling is disclosed, in particular, in the Analytical Protocol strategies at pages 7-8 and the protocol flow charts at pages 14 and 15 of the Provisional Application.

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9. Support for the invention is also found in the priority document U.S. Provisional Patent Application 60/208,184, filed May 31, 2000. For example, at page 26, lines 6-17, isotopic labeling of proteins is disclosed, and at page 6, lines 17-30, and at page 29, lines 9-11, for example, isotopic labeling of peptides produced by proteolysis of a protein is disclosed.

10. I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

November 17, 2003
Date

By: Fred E. Regnier
Fred E. Regnier, Ph.D.